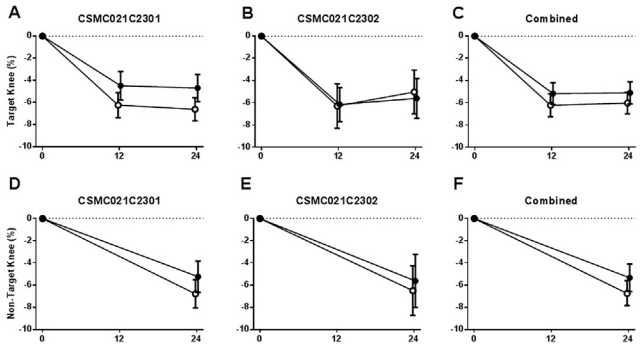
of this particular intervention may benefit a subgroup of OA patients, albeit further studies needs to validate this.



Change in total cartilage volume in % from baseline at month 12 and month 24 in target knee (A,B,C) and contra-lateral knee (D,E,F) in each of the studies and the studies combined. The figure shows the LS means and 95% CI; Closed circle: sCT; Open circle: Placebo

chemokine (C-C motif) ligand 5 (CCL5), cyclooxygenase 2 (Cox-2) and type X collagen were examined with immunostaining. Results were are expressed as the mean ± standard deviation. The differences between PC-treated and untreated groups were analyzed using Student's t test or Wilcoxon rank sum test.

Results: Meniscal calcification in the medial meniscus was absent in the partial-meniscectomy performed right knees. PC treatment significantly reduced the severity of cartilage degeneration and cartilage thinning in the partial-meniscectomy induced OA or non-calcification-

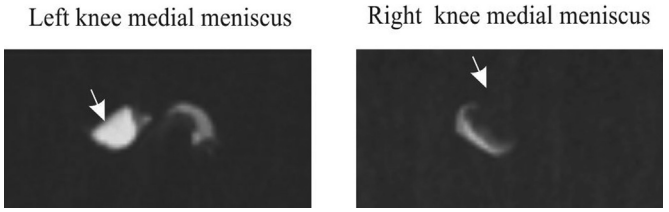


Figure 1. Radiographs of the medial meniscus. Left: Radiograph of medial meniscus of the left knee (non-operated knee) of the guinea pigs. The anterior horn was severely calcified or ossified (arrow). Right: Radiographs of medial meniscus of right knee (surgery performed knee) of the guinea pigs. The severely ossified anterior horn was absent, removing by the partial-meniscectomy surgery (arrow).

MRI subpopulation: Demographic characteristics in intent-to-treat population [n(%) and mean (SD)]

	2301sCTN=278	2301PlaceboN=273	2302sCTN=155	2302PlaceboN=152	AllsCTN=433	AllPlaceboN=425
Sex - n(%)						
Male	84 (30%)	97 (36%)	63 (41%)	68(45%)	147 (34%)	165 (39%)
Female	194 (70%)	176 (64%)	92 (59%)	84 (55%)	286(66%)	260 (61%)
Age (years)	64.6 (6.9)	64.1(6.4)	65.0 (7.1)	64.4 (7.0)	64.8(7.0)	64.2 (6.6)
Race- n(%)						
White	277 (100%)	272 (100%)	155 (100%)	151 (99%)	432 (100%)	423 (100%)
Other	1 (0%)	1 (0%)	-(-)	1(1%)	1 (0%)	2 (0%)
BMI (kg/m^2)	29.0 (4.1)	28.7 (4.1)	28.6 (4.0)	28.6 (4.2)	28.8(4.1)	28.7 (4.1)
JSW (mm)[1]	3.38 (0.95)	3.35 (0.94)	3.31 (1.07)	3.48 (1.01)	3.35(0.99)	3.39 (0.96)
KL index n (%)[1]						
Grade 2	*241* (87%)	245 (90%)	121(78%)	125 (82%)	362 (84%)	370 (87%)
Grade 3	37 (13%)	28 (10%)	31 (22%)	27 (18%)	71 (16%)	55 (13%)
WOMAC pain (mm)[1]	233 (72)	242 (69)	238 (66)	237 (63)	237 (70)	241 (67)
WOMAC total (mm)[1]	1089(379)	1135 (376)	1014 (377)	993(370)	1062(379)	1084 (380)
Cartilage volume tibial (mm^3)[1]	2111 (503)	2196 (547)	2177 (559)	2200 (592)	2134 (524)	2198 (563)
Cartilage volume femoral (mm^3)[1]	4903(1118)	5110 (1134)	5087 (1108)	5132 (1142)	4969 (1117)	5118 (1136)
Cartilage volume total (mm^3)[1]	7013 (1543)	7305 (1599)	7264 (1590)	7332 (1652)	7103 (1563)	7314 (1617)

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PHOSPHOCITRATE REDUCED CARTILAGE DEGENERATION IN NON-CALCIFICATION INDUCED OSTEOARTHRITIS

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Purpose: It was believed that phosphocitrate (PC) exerted its disease modifying effect on calcification-induced osteoarthritis (OA) by inhibiting the formation of calcium crystals within the joints. However, recent studies suggest that PC exerts its disease modifying effect on OA, at least in part, through a crystal-independent mechanism. This study sought to investigate the disease-modifying effect of PC on partial-meniscectomy induced OA or non-calcification induced OA and test the hypothesis that PC is not only potentially a disease modifying drug for calcification-induced OA therapy but also potentially a disease-modifying drug for non-calcification-inducted OA therapy.

Methods: Male Hartley guinea pigs of 4 weeks old were subjected to intraperitoneal injections of PC and physiological saline respectively. Two months later, partial medial meniscectomy was performed on the right knee of all guinea pigs to remove the anterior horn (calcification site) of the medical meniscus. After the surgery, injections of PC and saline were resumed. Five months later, these guinea pigs were euthanized and hind limbs were collected. Meniscal calcification and cartilage degeneration was examined with digital x-ray, Indian ink and safranin O staining. Matrix metaloproteinse-13 (MMP-13), ADAM metallopeptidase with thrombospondin type 1 motif 5 (ADAMTS5), induced OA. The reductions in cartilage degeneration and cartilage thinning were accompanied with significantly decreased protein levels of MMP-13, ADAMTS5 and CCL5.

Conclusions: PC is not only potentially a disease modifying drug for calcification-induced OA therapy but also potentially a disease-modifying drug for non-calcification-inducted OA therapy. PC exerts its disease modifying activity on non-calcification-induced OA mainly by targeting the production of extracellular matrix-degrading enzymes through a crystal-independent mechanism. These findings provide further support for the development of PC or its analogues as disease-modifying drugs for human OA therapy.

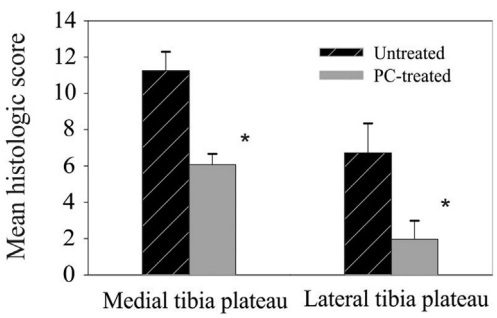

Figure 2. Mean histological scores of the tibia plateaus. Left bar group: Mean histological scores of the medial tibia plateaus of the rights knees in the untreated and PC-treated guinea pigs. Right bar group: Mean histological scores of the lateral tibia plateaus of the right knees in the untreated and PC-treated guinea pigs. * = P < 0.05 PC-treated versus the untreated guinea pigs.

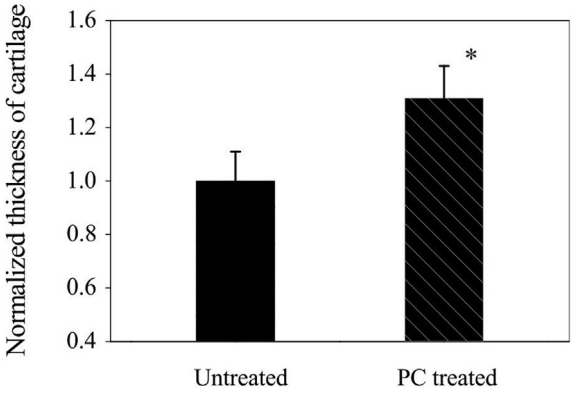

Figure 3. Cartilage thickness. Left: thickness of the medial tibia cartilage in the untreated guinea pigs. Right: thickness of the medial tibia cartilage in the PC-treated guinea pigs.

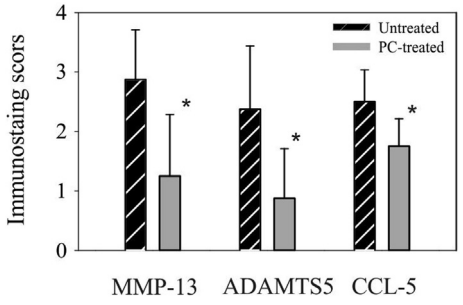

Figure 4. Immunostaining scores. Left bar group: MMP-15 immunostaining. Middle bar group: ADAMTS5 immunostaining. Right bar group: CCL-5 immunostaining.

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THE IN VIVO AND IN VITRO EFFECT OF INHIBITORS OF BRD4 AND CDK9 ON EARLY PHASE OF POST TRAUMATIC OSTEOARTHRITIS

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Purpose: Joint trauma is a risk factor for osteoarthritis (OA), and about 50% of patients with ACL or meniscal injury develop posttraumatic OA (PTOA) within 10-20 years. The acute response to joint trauma increases transcription of pro-inflammatory cytokines and proteinases such as matrix metalloproteinases (MMPs), which trigger the onset of OA changes. Bromodomain protein 4 (Brd4) and cyclin-dependent kinase 9 (CDK9) control the rate-limiting step of the transcription of primary response genes, including most pro-inflammatory genes, by positively regulating mRNA elongation with phosphorylating and releasing the RNA polymerase II. The purpose of this study is to investigate the effects of small molecule inhibitors of Brd4 (JQ1) and CDK9 (Flavopiridol) on the activation of inflammatory genes using chondrocytes and cartilage tissue under inflammatory stimuli, and a mouse PTOA model.

Methods:

• Treatment of chondrocytes Human chondrocytes were cultured 5 hours with inflammatory stimuli (either 10ng/ml IL1b, 10ng/ml TNFa, or 100ng/ml IL6 and 60ng/ml IL6 receptor), with or without drugs. Treatment conditions were: 1) vehicle only (Ctrl), 2) no drug with cytokine, 3) Hi JQ1 (1200nM) with cytokine, 4) Hi Flavopiridol (250nM) with cytokine, 5) Combination of Lo JQ1 (250nM) and Lo Flavopiridol (60nM) with cytokine. Total RNA was extracted and analyzed by real time RT-PCR and microarray.

• Treatment of cartilage explants
Bovine cartilage explants were isolated and randomly assigned to 5 groups cultured as described above, with the cytokine being 10ng/ml IL1b. Glycosaminoglycan (GAG) released into the culture media was measured.

• PTOA animal model
The right knees of mice were injured with a mechanical compression, which causes rupture of the anterior cruciate ligament and leads to PTOA. Immediately after injury, mice were treated daily with JQ1 and/or Flavopiridol. Treatment conditions were: 1) vehicle only (Ctrl), 2) Hi JQ1 (50mg/kg), 3) Hi Flavopiridol (7.5mg/kg), 4) Combination of Lo JQ1 (17mg/kg) and Lo Flavopiridol (2.5mg/kg) Flavopiridol.

• MMPSense Assay
To assess MMP activity, MMPSense 750 was injected to mice and the intensity of signal from the MMPSense in the knees was measured by in vivo imaging.

Results:

• The mRNA expression levels of pro-inflammatory genes (iNOS, Cox2) and catabolic genes (MMP-1, -3, -9, and -13, and ADAMTS4) were significantly induced by all 3 inflammatory cytokines, and this induction was suppressed by all 3 drug treatments. The combination of both drugs at lower doses suppressed gene expression similarly or more strongly than single high doses of each individual drug.

• Microarray showed that expression, 873 genes were induced >1.5-fold by IL1b compared to baseline. IL-1b treatment in the presence of either JQ1 or Flavopiridol alone prevented the induction of many genes. However, a combination of both drugs prevented the induction of most IL-1b response genes. (Fig.1)

• IL1b treatment of cartilage explants induced significant release of GAG within 3-6 days. GAG release was effectively prevented when IL-1b treatment in the presence of either or both drugs. (Fig2)

• In PTOA mouse model, knee injury caused significant increases of IL1b and IL6 expression in the injured joint. All 3 treatments showed effect to prevent increases of these cytokines and drug combination was more effective than single drugs. (Fig3A, B) MMP activity in injured knee was suppressed by all 3 treatment similarly at 24h and 48h after injury. (Fig3C, D)

Conclusions: JQ1 and Flavopiridol are each able to effectively repress a panel of pro-inflammatory and catabolic genes in chondrocytes induced by inflammatory stimulus. We found that the combination of the 2 drugs showed a synergistic interaction, with similar or better repression achieved at reduced drug doses. Although previous reports indicated that Brd4 and CDK9 control mRNA transcription by regulating a common checkpoint, microarray analysis showed that there were also inflammatory genes only affected by each drug individually. Ex vivo and in vivo study also demonstrated the combination of lower dose of the drugs has similar or better intensity of effect. This indicates that using both drugs together may be able to suppress inflammation after trauma with preventing side effects induced by overdose, leading to novel treatment for PTOA.